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Studies and Results

Task I. Characterization of MT-DNIIR-27 and MT-DNIIR-4 mice

Analysis of Intact MT-DNIIR-27 mice:

Tissues from MT-DNIIR-27 and MT-DNIIR-4 mice aged from 6 to 54 weeks have been collected and analyzed for pathological changes. These analyses have demonstrated that MT-DNIIR mice undergo pathological changes that bear striking resemblance to prostatic intraepithelial neoplasia (PIN) observed in the human prostate. The pathological characterization of these tissues has been performed in consultation with a pathologist (Dr Scott Shappell) in a blinded study comparing the MT-DNIIR mice to age matched wild type control mice. The definitions of low and high grade PIN are the same as those used to classify these neoplastic changes in human prostatic tissue. These include the development of nuclear atypia, nuclear stratification, chromatin structure alterations and changes in the overall architecture of the prostate in these mice. Tissues for both MT-DNIIR-27 and MT-DNIIR-4 mice have been collected, however the MT-DNIIR-27 have been characterized more completely than those collected from the MT-DNIIR-4 mice and their analysis is described below.

In untreated MT-DNIIR-27 mice, regions of focal high grade (HG)-PIN are observed in the lateral prostate (LP) of mice at 16 weeks of age. These neoplastic changes continue to be observed in the LP up to 46 weeks of age (oldest animals analyzed). Similarly, the ventral prostate (VP) of these mice develops extensive low grade (LG)-PIN associated with focal regions of HG-PIN at 16 weeks of age, which progresses to multi-focal and extensive HG-PIN, by 46 weeks. In contrast to the LP and VP, the dorsal prostate (DP) of these mice develops only focal LG-PIN which does not progress to HG-PIN even by 49 weeks of age.

MT-DNIIR-27 mice treated with zinc sulfate (25mM supplied in the drinking water) show similar changes in the DP and the LP, however the ventral prostate shows more pronounced changes compared to the VP of untreated MT-DNIIR-27 mice. There is more significant nuclear stratification, greater nuclear atypia and the development of macronucleoli. The development of extensive LG-PIN is observed at 15 weeks in these mice, which progresses to multi-focal HG-PIN by 19 weeks of age. Extensive HG-PIN, involving greater than 75% of all glands in the VP, is observed by 29 weeks of age. Currently tissues from mice aged 42 weeks are being collected and will be analyzed for pathological changes.

Histological analysis of these mice is currently underway and preliminary results indicate that the number of cells positively staining for PNCA is not greatly different to that observed in wild type age matched controls. However these results require closer scrutiny and quantitative analysis before a final conclusion can be reached. Analysis of the level of apoptosis in these tissues is currently being performed and it will be interesting to determine if the level of apoptosis has decreased as a result of loss of functional TGFβ signaling.

Analysis of Castrated MT-DNIIR-4 and MT-DNIIR-27 mice:

Preliminary studies examining the effect of castration on the prostate of MT-DNIIR-4 mice revealed that by 7 days post castration, the DP and LP had not regressed. Histopathological analysis of these tissues revealed that epithelial cell

height had had not changed by 7 days post-castration whilst wild-type control mice had decreased epithelial cell height (as would be expected after androgen withdrawal). Staining for PCNA revealed that the DP and LP of MT-DNIIR-4 mice 7 days post-castration were undergoing a significant increase in proliferation. This increase in proliferation was not observed in the VP or the anterior prostate (AP), nor in any prostatic tissues of wild-type control mice. The level of proliferation is significantly greater that than of sham operated age matched MT-DNIIR-4 mice. Similarly, MT-DNIIR-27 mice show the same histopathological profile after castration, as do the MT-DNIIR-4 mice. The LP shows a sharp increase in the number of PCNA positive cells at 7 days post castration. Analysis of MT-DNIIR-27 tissues from mice up to 10 weeks post castration show that at approximately 14 days after androgen withdrawal the prostate begins to regress, however the rate of regression is slower than that of wild-type mice. The reason for this is not clear, however Western blot analysis is currently being performed to determine if the level of the DNIIR transgene is decreasing, and as a result the endogenous TβRII levels are increasing. Although the mice are being maintained on zinc sulfate, the levels of the transgene may be decreasing due to a decrease in the levels of the zinc channel (which is androgen regulated [personal communication Dr R. Franklin]).

Task II. MT-DNIIR × LPBTAg Transgenic Lines

MT-DNIIR-4 mice have been crossbred to LPB-12T-7f mice in three separate experiments. In all cases, the size of the litter was small, and screening of the mice to determine which transgene was expressed revealed that no mice were bi-genic for both the DNIIR and the LPBTag transgenes. As a result we have decided to cross the MT-DNIIR-27 mice with the LPB-12T-7f mice. Currently we have obtained bi-genic mice from this crossbreeding and are aging the mice to begin collecting samples.

Task III. Characterization of LPB-DNIIR mice

Initially, I proposed generating LPB-DNIIR mice using the 12Kb probasin promoter which has been demonstrated by the Matusik laboratory to direct high levels of transgene expression to the prostatic epithelium. Since submitting the grant proposal we have published a study showing that the composite minimal probasin promoter ARR_2PB (Zhang et al, 2000 in press) can generate equally high levels of prostate epithelial specific transgene expression and more importantly that this promoter generates greater numbers of high expressing founder lines. As a result I have generated ARR_2PB-DNIIR transgenic mice. At this time three separate founder lines have been established. Preliminary pathological analysis indicates that all three mice show focal regions of nuclear atypia with minimal to mild nuclear stratification in the ventral prostate. Currently mice are being bred and aged for tissue collection. At present, Western blot analysis is being performed to determine which of the lines is the highest expressing and may therefore be the best choice for further analysis.

Publications

EXPRESSION OF A TRUNCATED, KINASE DEFICIENT TGFβ TYPE II RECEPTOR IN THE MOUSE PROSTATE. Oral Presentation#19-3, The Endocrine Society 81st Annual Meeting, 1999.

DISRUPTION OF THE TGFβ PATHWAY IN TRANSGENIC MICE PREVENTS CASTRATION-INDUCED PROSTATIC REGRESSION. Poster #123, American Urologic Association 95th Annual Meeting, 2000.

Two manuscripts are currently being prepared describing the work completed in Task I.

DISRUPTION OF THE TGFβ PATHWAY IN TRANSGENIC MICE PREVENTS CASTRATION-INDUCED PROSTATIC REGRESSION.

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Early reports have noted increased expression of TGFβ1 and loss of TGFβ type I and type II (TβRII) expression with increasing prostate cancer grade. We have generated mice (MT-DNIIR) that express a metallothionein promoter driven truncated TβRII which results in a dominant negative mutant that blocks the TGFβ pathway in the prostate. Two founder lines (MTR4 & MTR27H) revealed focal regions of low grade PIN, which progress to high grade PIN. Aged mice showed significant histological changes including the development of cribiform and invasive cancer (unpublished data). The MT-DNIIR mice show histopathologic changes mimicking those observed in human prostate cancer. Since TGFβ is negatively regulated by androgens, and has been implicated as a mediator of castration-induced cell death in the prostate, we examined the MTR4 and MTR27H mice to determine the effects of loss of TGFβ signaling in the regressing prostate. MTR4 and MTR27H mice were castrated and maintained on zinc sulfate in the drinking water. Prostates were collected, weighed and examined histologically, and immunostaining for Proliferating Nuclear Cell Antigen (PCNA) was performed. In non-transgenic mice the anterior prostate (AP), dorsolateral prostate (DLP) and ventral prostate (VP) regressed to 13% (AP), 18% (DLP) and 11% (VP) of sham operated controls (100%) by 35 days post-castration. The histology associated with these tissues was consistent with that expected after androgen withdrawal and no positive immunostaining for PCNA was observed. In contrast, the prostates from 35 day post-castration MTR4 and MTR27H mice had regressed to: AP 30% and 34%, DLP 81% and 57% and VP 50% and 33% respectively. The DLP of both mouse lines showed glands containing normal tall, columnar epithelium beside other glands that were clearly atrophic, suggesting that the level of transgene expression is variable throughout the prostate. PCNA immunostaining was observed in the prostates of sham operated MTR4 and MTR27H mice. At 7 days post-castration, PCNA staining was also observed in the DLP of MTR4 and MTR27H mice, however, by 35 days post-castration no PCNA immunostaining could be detected. Although the origin of androgen independent prostate cancer in humans remains unknown, this data suggests that disruption of the TGFβ pathway may be a contributing event to the development of androgen independent disease through the prevention of complete prostatic regression after androgen ablation.

EXPRESSION OF A TRUNCATED, KINASE DEFICIENT TGFβ TYPE II RECEPTOR IN THE MOUSE PROSTATE.

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Early reports have noted increased expression of TGFb1 and loss of TGFb receptor type I (TbRI) and II (TbRII) expression with increasing cancer grade in human prostate cancer (PCa). Although the TGFb superfamily has been implicated in the progression of human PCa, it's role in the development and progression of this disease has not been addressed in transgenic animal models. We have generated transgenic mice that express a metallothionein (MT) promoter driven truncated TbRII which results in a dominant negative (DN) mutant that can form a heteromeric complex with the endogenous TbRI. The prostates from two different founder lines (MT-DNIIR-4 and MT-DNIIR-27) were examined for transgene expression by *in situ* hybridization and for histopathological changes associated with loss of TGFb function. *In situ* hybridization, using a transgene specific cDNA probe, showed transgene expression in the ventral (VP), dorsolateral (DLP) and anterior prostate (AP). The expression of the transgene in MT-DNIIR-27 mice was low at 7 weeks but increased by 16.5 weeks of age. Transgene expression was similar in MT-DNIIR-4 mice, however the levels were higher. Histological examination of MT-DNIIR-27 prostates revealed focal changes in prostatic morphology at approximately 12 weeks of age that are comparable to low grade prostatic intraepithelial neoplasia (LGPIN) in humans. By 16.5 weeks of age regions of high grade prostatic intraepithelial neoplasia (HGPIN) are present in all animals examined. MT-DNIIR-4 mice at 33 weeks show regions of HGPIN with local invasion, increased thickening of the basement membrane/fibromuscular stroma, as well as regions of cribriform and solid architecture accompanied with comedo necrosis which is reminiscent of intraductal carcinoma (IDCa). IDCa is believed to represent the intraductal spread of established carcinoma, and has been correlated with higher Gleason grade, higher tumor volume and poor prognosis. Therefore the MT-DNIIR mice show pre-neoplastic changes mimicking those observed in human prostate disease. Although the origin of human premalignant lesions remains unknown, this data suggests that disruption of the TGFb pathway may be the initiating event(s) in mouse and human prostatic neoplasia.